

THE INNER EAR IS RESPONSIBLE FOR DETECTION OF INFRASOUND IN THE PERCH (*PERCA FLUVIATILIS*)

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Summary

In a previous study of infrasound detection in the cod, the inner ear was suggested to be the sensory organ responsible for the responses. However, a possible involvement of the lateral-line system in the observed low-frequency detection could not be ruled out. The infrasound sensitivity was therefore studied in perch (*Perca fluviatilis*) with normal and blocked lateral-line organs. The experiments were performed using a standing wave acoustic tube and the cardiac conditioning technique. All perch readily responded to infrasound frequencies down to 0.3 Hz with threshold values of approximately $2 \times 10^{-4} \text{ m s}^{-2}$. These thresholds were not affected by complete blocking of the lateral-line system with Co^{2+} , which suggests that the inner ear is responsible for the observed infrasound detection by the perch.

Introduction

Wolff (1967) was the first to establish an audiogram for perch (*Perca fluviatilis*). Although both the stimulation and recording techniques of his behavioural study were rather imprecise, Wolff (1967) obtained a well-defined upper frequency limit of about 300 Hz in the perch. This finding was later supported by Sand (1973), who measured saccular microphonic potentials during horizontal vibration of perch at different frequencies in air.

Because of limitations in his stimulation technique, which was based on underwater loudspeakers suspended in a small tank, Wolff (1967) was unable to test reactions to very low frequencies. One of his fish, however, showed clear responses to 30 Hz sound stimulation, which was the lowest frequency employed. Sand (1973) obtained microphonic responses to 35 Hz vibrations. Even in species which utilize the swimbladder as an accessory hearing organ, the relevant inner ear stimuli in the low-frequency range are the particle accelerations in the incident sound field (Chapman and Sand, 1974; Sand and Enger, 1973; Sand and Hawkins, 1973). When the relative vibrogram from Sand (1973) is plotted in terms of acceleration, there is a mere 7.4 dB decrease in sensitivity at 30 Hz compared with

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120 Hz. This indicates that the acoustic sensitivity of the perch may extend well into the infrasound range.

The sensitivity of fish to infrasound has so far only been tested in the Atlantic cod (*Gadus morhua*). Sand and Karlsen (1986) found an acute sensitivity of this species to frequencies below 10 Hz, with thresholds close to 10^{-5} m s^{-2} at 0.1 Hz. The tests were performed using a specially designed acoustic tube (Hawkins and MacLennan, 1976; Sand, 1981) in which the fish being tested was loosely suspended in the water column filling the tube. The stimulation, which consisted of vibrations of the whole water column including the fish, is not expected to activate the lateral-line organs significantly. For this reason it was suggested that the inner ear accounted for the infrasound thresholds in cod. However, an involvement of the lateral line could not be ruled out. Infrasound is an important component of the ambient background noise in the sea, and Sand and Karlsen (1986) suggested that detection of infrasound may be utilized for orientation in migrating fish. Furthermore, swimming fish produce predominantly low-frequency hydrodynamic noise below 20 Hz, and high sensitivity in this frequency range may be important for both prey and predatory fish (Enger *et al.* 1989).

Recently, Denton and Gray (1983, 1988, 1989), Kroese and Schellart (1987) and Kalmijn (1988, 1989) have shown that lateral-line organs in fish respond to local water accelerations or velocity rather than to displacement as concluded by Harris and van Bergeijk (1962). Moreover, replotting existing neurophysiological response curves in terms of the proper stimulus parameter (acceleration) reveals lateral-line tuning curves with low-pass characteristics and an optimal frequency range that may extend well into the infrasound region (see Kalmijn, 1988, 1989). This is also in accordance with behavioural responses in fish, indicating that the lateral-line is primarily sensitive to low-frequency flow fields (Dijkgraaf, 1963; Montgomery and MacDonald, 1987; Enger *et al.* 1989; Montgomery, 1989). Both the inner ear and the lateral-line system in fish would therefore seem suitable for detecting water motions in the infrasound range.

As an experimental tool to investigate the relative importance of the lateral-line sensory organs and the inner ear in various types of fish behaviour, Karlsen and Sand (1987) developed a method for selective blocking of the lateral-line system in freshwater fish. They showed that, by adding small amounts of Co^{2+} to the external water, it was possible to block completely the lateral-line organs in roach (*Rutilus rutilus*) while leaving the inner ear unaffected. The blocking effect of Co^{2+} was counteracted by Ca^{2+} , and this method is therefore not applicable in sea water, which contains about $10 \text{ mmol l}^{-1} \text{ Ca}^{2+}$. In the present investigation, this pharmacological method was used to test whether the lateral-line organs are involved in the detection of homogeneous infrasound vibrations in fish. The experimental arrangement was essentially the same as in the study on cod (Sand and Karlsen, 1986) but, because of the limitations of the pharmacological method, a freshwater species was selected as experimental animal. The infrasound detection tests were therefore performed on control perch and on perch in which the lateral-line system had been blocked by Co^{2+} .

Materials and methods

Animals

Perch (*Perca fluviatilis* L.) (18–20 cm) were caught in gill nets and gently transported to the test site. All fish were kept in large tanks with dechlorinated tap water (8–10°C) for at least 2 weeks before being used in experiments. During this adaptation period, the fish fed on the regularly supplied insect larvae and dry pellets.

Electrocardiogram recording and infrasound stimulation

Heart rate was continuously recorded throughout the experiments by means of small cardiac electrodes attached to the fish during light MS-222 anaesthesia. While still anaesthetised, the fish were gently placed inside a neutrally buoyant plastic netting cage, which was held loosely in the centre of the tube by a fine string. The loose string prevented extensive rolling of the cage, but did not affect the horizontal movements of the fish and its cage along with the water column in the tube.

The experimental set-up was essentially the same as that described by Sand and Karlsen (1986). In short, an aluminium acoustic tube was fitted at each end with a rubber membrane connected to a piston and a vibrator. Large and uniform particle movements throughout the length of the tube were generated by driving the vibrators 180° out of phase. In the low-frequency range, the particle movements in the tube closely followed the movements of the pistons, which were measured by linear variable differential transformers [Shaevitz, 100 DC-D, frequency response d.c. to 500 Hz (–3 dB) and sensitivity 4 mV μm^{-1}]. Particle motions in this report are presented as the root-mean-square acceleration of the pistons.

The dynamic characteristics of the acoustic tube were comparable to that of an underdamped mechanical second-order system with a damping coefficient of 0.36 and a natural frequency of 27 Hz. The lowest frequencies tested in the present experiments were 0.1 Hz and 0.3 Hz. At these frequencies, the spring forces of the system dominated over the inertial and friction forces, and the displacement of the water column in the acoustic tube therefore closely followed the voltage waveform supplied to the vibrators. The driving waveform at these frequencies consisted of 2–5 sine wave cycles initiated at zero current and d.c. shifted one peak value (see Fig. 2). The acceleration of the water column then described a normal sine wave, which started at its peak value. There were no overshooting on-transients (Sand and Karlsen, 1986), but the calculated Fourier spectrum for the signal showed the presence of higher-order frequency components of decreasing amplitude. The components one octave on either side of the actual stimulus frequency were, however, reduced by more than 10 dB. They would, therefore, be insignificant at low stimulation intensities close to threshold.

Above 0.3 Hz the stimulus waveform was a pure sine wave with no offset. To avoid on-transients at these frequencies, the rise time of the stimuli covered several cycles (see Fig. 2). The rise in stimulus amplitude was produced by feeding

a ramp signal to a voltage-gated amplifier preceding the attenuator and filter. A linear tapering of waveform amplitude is a standard technique in Fourier analyses to prevent spectral leakage, and the presence of higher-order frequency components in the stimulus should thus be minimal.

Conditioning

All test fish were allowed to recover for 12–24 h in the acoustic tube before their sensitivity to infrasound was tested employing the cardiac conditioning technique (Chapman and Hawkins, 1973). A sound stimulus lasting 10–20 s was presented to the fish and immediately followed by a mild electric shock to the tail region. The conditioned fright response was a significant decrease in the heart rate. Normally, even the first trial resulted in a pronounced bradycardia, and the electric shock apparently merely prevented habituation to the response. Initially the conditioned response was established by 3–5 trials at a high stimulus level of 0.1 m s^{-2} or approximately 60 dB above the sensitivity found in the Atlantic cod (Sand and Karlsen, 1986). The threshold level was subsequently determined by reducing the stimulus intensity in steps of 3–6 dB. Close to the threshold several 3-dB up and down steps were performed, and the threshold was calculated as the stimulus level giving 50 % probability of a positive response (Dixon, 1965). Because of the limited working range of the vibrators, a starting stimulus intensity of 0.1 m s^{-2} was not possible at 1 Hz and below. Testing at these frequencies therefore began at the maximum intensity obtainable, which was $2 \times 10^{-2} \text{ m s}^{-2}$ at 1 Hz, 10^{-3} m s^{-2} at 0.3 Hz and $8 \times 10^{-5} \text{ m s}^{-2}$ at 0.1 Hz.

The time between each trial varied randomly between 5 and 30 min. A test was only initiated if the heart rate had been regular for at least 2 min, i.e. the time between heart beats stayed within 10 % of the mean interval. A response was considered positive when a heart beat interval during the sound stimulus exceeded the longest of the 20 prestimulus intervals by at least 10 %, corresponding to a probability of less than 0.01 (*t*-test). The bradycardia responses observed in the experiments were normally much more pronounced than this, and it was independent of stimulus strength above threshold. Rigorous statistical testing to reveal significant changes in heart rate during the stimulus period was therefore not needed.

Lateral-line sensitivity and Co^{2+} treatment

The effect of Co^{2+} on the lateral-line organs was examined by exposing a group of perch to normal fresh water containing $0.1 \text{ mmol l}^{-1} \text{ Co}^{2+}$ and less than $0.05 \text{ mmol l}^{-1} \text{ Ca}^{2+}$ for 12 and 24 h according to the method described by Karlsen and Sand (1987). Perch kept in the normal fresh water without Co^{2+} served as controls. After the exposure period, the lateral-line sensitivity was determined by placing amytal-anaesthetized fish (Keys and Wells, 1930) side down on the angled bottom of a chamber recirculated with the test solution (Fig. 1A). Multiunit lateral-line nerve activity was then recorded by platinum electrodes connected to a preamplifier, a window discriminator and a pulse counter reset at 0.5 s intervals.

The lateral-line organs were stimulated by a water jet delivered from a Pasteur pipette. This represents an extreme stimulation of the neuromasts, and the lateral-line system was considered to be completely blocked when even strong stimulation caused no increase in firing frequency (Karlsen and Sand, 1987).

Results

Effect of Co^{2+} treatment

The effect of $0.1 \text{ mmol l}^{-1} \text{ Co}^{2+}$ on the lateral-line organs in a perch is illustrated in Fig. 1B,C. After 12 h exposure to water containing Co^{2+} , at a Ca^{2+} concentration of less than 0.05 mmol l^{-1} , even strong pipette stimulation had no effect on lateral-line nerve activity (Fig. 1C). This shows that Co^{2+} can be used as an effective blocker of the lateral-line system in perch in the same manner as has previously been shown for roach (Karlsen and Sand, 1987), bluegills (*Lepomis macrochirus*) (Enger *et al.* 1989) and Mexican cave fish (*Anoptichthys jordani*) (Abdel-Latif *et al.* 1990). At the recording site, the lateral-line nerve contains fibres innervating both canal and superficial neuromasts. The multiunit activity recorded therefore included both these types of sensory structures.

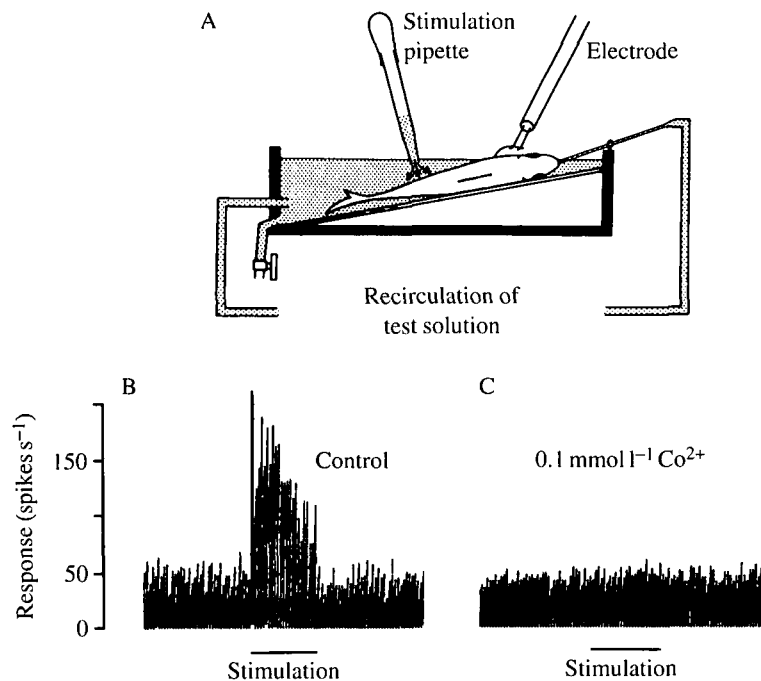


Fig. 1. Multiunit lateral-line nerve recordings from superficial and canal neuromasts in perch. (A) Diagram of the experimental apparatus. (B) In control fish, a water jet from a Pasteur pipette directed at the lateral line caused a large increase in firing frequency. (C) After 12 h in water containing $0.1 \text{ mmol l}^{-1} \text{ Co}^{2+}$ and less than $0.05 \text{ mmol l}^{-1} \text{ Ca}^{2+}$, the response of the lateral-line organs was completely blocked. Stimulation lasted 10 s.

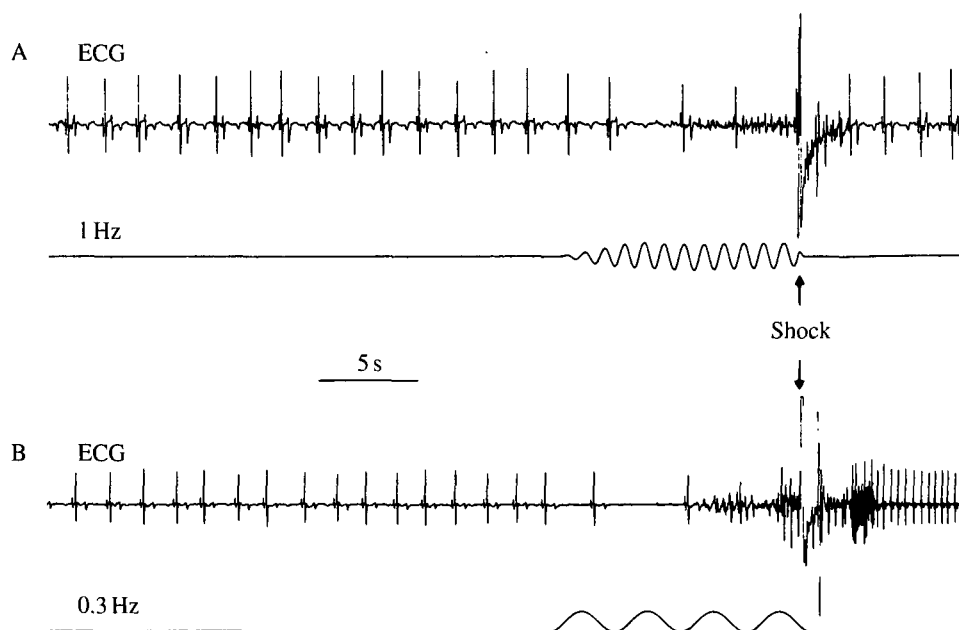


Fig. 2. Typical conditioned cardiac responses (slowing of the heart rate, ECG) to 1 Hz (A) and 0.3 Hz (B) infrasound stimulation. The lower trace in each example is a record of the output from the linear variable differential transformer and shows the piston displacement. An electric shock was given at the end of each stimulus. Record A is from a control perch while record B shows the response of a perch in which the lateral-line system had been completely blocked by $0.1 \text{ mmol l}^{-1} \text{ Co}^{2+}$. The stimulation intensity was 8 dB above threshold in each example. The increased electric noise in the recordings towards the end of stimulation was caused by movements of the fish anticipating the shock.

Response to infrasound

All of the eight control and three Co^{2+} -treated perch were easily conditioned to infrasound down to 0.3 Hz. Only two of the fish (both control animals) responded to 0.1 Hz at the maximum stimulation intensity of $8 \times 10^{-5} \text{ m s}^{-2}$ possible at this frequency. In both groups of fish the best responses, i.e. large bradycardia and stable electrocardiogram (ECG) between tests, were obtained in the first 2 days after the training to infrasound had begun. Within this period it was possible to obtain threshold values for 2–4 frequencies. The threshold for a given frequency was determined 2–3 times. The differences between these values were less than 6 dB in all cases, and the average was taken as the final threshold. Typical responses to infrasound stimulation are shown for 1 Hz and 0.3 Hz in Fig. 2. The bradycardia responses were usually large and did not decrease at low stimulation intensities approaching threshold, making it easy to decide whether a response was positive.

The threshold values obtained at 0.3 Hz, 1 Hz and 3 Hz were all approximately $2 \times 10^{-4} \text{ m s}^{-2}$ as shown with filled symbols in Fig. 3. The thresholds at 10 Hz and 30 Hz were elevated. The open symbols in Fig. 3 show individual thresholds from

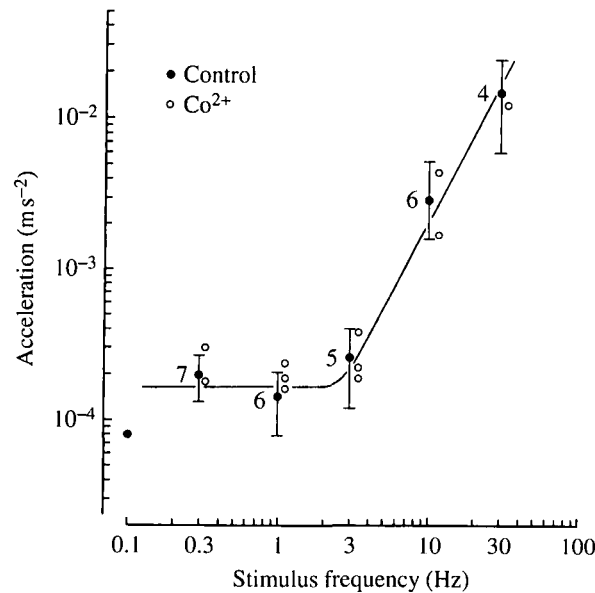


Fig. 3. Acceleration thresholds (mean \pm s.d.) for control (filled circles) and Co^{2+} -treated (open symbols) perch stimulated at different frequencies. The numbers of thresholds obtained at each frequency for control fish are indicated. Each open symbol represents an individual threshold. The line was fitted by eye.

perch exposed to $0.1 \text{ mmol l}^{-1} \text{ Co}^{2+}$. Blocking the lateral-line system did not affect the sensitivity to the infrasound stimulation.

Discussion

In the present experiments perch clearly responded to infrasound down to 0.3 Hz. The thresholds at 0.3 Hz, 1 Hz and 3 Hz were all approximately $2 \times 10^{-4} \text{ m s}^{-2}$ or 94 dB below the acceleration due to gravity. This sensitivity is comparable to the corresponding infrasound thresholds found in the Atlantic cod (Sand and Karlsen, 1986). Two of eight tested control perch also responded to 0.1 Hz at the maximum possible stimulation intensity of $8 \times 10^{-5} \text{ m s}^{-2}$ at this frequency. This intensity, which was clearly at the detection limit for perch, was 20 dB above the corresponding threshold in the cod and indicates a significant difference in sensitivity between the two species at this frequency. The shape of the infrasound audiogram found in cod was, however, probably influenced by the background noise at the test site (Sand and Karlsen, 1986). This may also have been the case in the present study. The vertical vibrations of the acoustic tube measured in one-third octave bands were between 10^{-6} and 10^{-5} m s^{-2} in the frequency range 0.3–8 Hz, which was similar to the previous study on cod. Above 8 Hz the background noise increased steadily, reaching maximum values of $8 \times 10^{-4} \text{ m s}^{-2}$ at 100 Hz. Above 100 Hz the noise declined to approximately

10^{-4} m s^{-2} at 1 kHz. The reduced sensitivity observed in perch at 10 Hz and 30 Hz may reflect the increase in background noise at these frequencies. In addition, the resonant frequency of the acoustic tube was 23 Hz, so that horizontal background movements of the water column in the tube were accentuated around this frequency. This was also apparent by watching the amplified outputs from the linear variable differential transformers between stimulations. The thresholds at 10 Hz and 30 Hz were therefore probably masked.

The lateral-line system in fish has an important role in detecting local, low-frequency water movements when the fish is extremely close to the source (see Dijkgraaf, 1963; Sand, 1984). Since the cupula of the lateral-line organs have a density close to the density of water (Jielof *et al.* 1952), only water movements relative to the fish surface will stimulate the lateral-line organs (Sand, 1981). In the present experiments the fish were accelerated together with the surrounding water, which simulated natural sound stimulation in the field at some distance from the source. For the detection of such infrasound stimuli, where the relative water movements are believed to be extremely small, the inner ear is therefore probably the sensory organ involved. Moreover, complete blockage of the lateral-line organs by Co^{2+} did not affect the infrasound thresholds, demonstrating that the observed infrasound responses in perch were not dependent on the lateral line.

The audible frequency range has been studied in several fish species (Fay, 1988). A common feature of these audiograms is a fairly sharp and well-defined upper frequency cut-off which, in most species, is below 1 kHz. In the low-frequency region the picture is more complicated. However, when thresholds are presented as acoustic pressure or particle displacement, which has been common practice, all audiograms show a reduced sensitivity below approximately 50 Hz. This has led to the general notion that the audible frequency range in fishes is rather narrow, and that lower frequencies are of little functional importance. This may not, however, be the case. In audiograms expressing low-frequency thresholds as acceleration, which is more likely to be the proper stimulus parameter (Lewis, 1984; Sand and Karlsen, 1986; Kalmijn, 1989), the apparent sensitivity drop at low frequencies is abolished. Accordingly, in the present study the audible frequency range of perch has been extended to at least 0.3 Hz, indicating that infrasound may represent a behaviourally significant stimulus to fish.

The shape of the acceleration stimulus at the two lowest frequencies tested in this study was a sine wave starting at its peak value. The initial stimulus period was therefore contaminated with higher-order frequency components of reduced amplitude. The limited working range of the vibrators used also reduced the possibilities for conditioning at 0.1 Hz. Additional experiments were therefore designed in order to avoid these limitations (Karlsen, 1992).

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